

PROJECT NUMBER: 6902
PROJECT TITLE: Biochemical Special Investigations
PROJECT LEADER: B. D. Davies
PERIOD COVERED: June, 1989

I. NICOTINE SPECIFIC MONOCLONAL ANTIBODY

- A. **Objective:** To obtain a monoclonal antibody (MCA) against nicotine (NIC-MCA).
- B. **Results:** Preliminary screening of spent media from 14 clones was completed using standard assay procedures. Several were shown to react specifically with nicotine coupled to thyroglobulin. None of these interactions was inhibitable by nicotine (1×10^{-4} M) under the standard conditions. A modification to the standard procedure, involving increased nicotine concentrations and dilutions of the spent media (antibody), has been introduced and has increased the sensitivity of the assay. Preliminary assessment of data from assays using the modified protocol do show moderate levels of nicotine inhibition of binding.
- C. **Plans:** Further examine the sensitivity of the modified screening assay and quantitate the nicotine inhibition of binding of spent culture media from clones. Implement modified assay to fully screen clones.
- D. **References:**
Crockett, E. Notebook No. 8783, p. 100.

II. ADDITIONAL APPROACHES TOWARD PUTRESCINE METHYLTRANSFERASE (PMT) ISOLATION

- A. **Objective:** Provide additional experimental approaches to assist in the effort to isolate PMT.
- B. **Results:** An ELFE fractionation was performed on a mixture of PMT enzyme active material and photoaffinity labeled ^3H -PMT. The bulk of the PMT enzyme activity was recovered in fractions 7 and 8, mimicking previous results from our Project and Project 1902. Protein bound ^3H was also recovered in fractions 7 and 8. This indicated that under native electrophoresis separation procedures ^3H -PMT and native PMT migrate coincidentally.

An experiment was performed to examine the total level of incorporation of SAM in PMT/PAL experiments utilizing a high specific activity/low concentration versus a low specific activity/high concentration ^3H -SAM solutions. The results of that experiment indicated that more SAM was incorporated into PMT using low specific activity solutions. However, as expected, lower levels of ^3H were incorporated.

- C. Plans: Repeat the ELFE experiment and determine if larger quantities of ^3H -PMT can be obtained. Prepare larger quantities of the low specific activity, ^3H -SAM, PAL/PMT material and determine if less ^3H is lost during subsequent fractionation procedures.

D. References:

Crockett, E. Notebook No. 8783, p 100.
Dunn, R. Notebook No. 8721, pp. 120-140.